DATE: 03/28/2002



PCT09

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TIME: 10:45:19 PATENT APPLICATION: US/09/980,585A

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1 <110> APPLICANT: LILIUS, Esa-Matti

VIRTA, Marko

3 <120> TITLE OF INVENTION: A Method to Enable Assessment of Growth and Death of Microorganisms

4 <130> FILE REFERENCE: 2328-124

5 <140> CURRENT APPLICATION NUMBER: US/09/980,585A

C--> 6 <141> CURRENT FILING DATE: 2000-06-07

7 <150> PRIOR APPLICATION NUMBER: PCT/FI00/00507

8 <151> PRIOR FILING DATE: 2000-06-07

9 <150> PRIOR APPLICATION NUMBER: FI 991296

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11 <160> NUMBER OF SEQ ID NOS: 4

12 <170> SOFTWARE: PatentIn version 3.0

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15 <211> LENGTH: 5051

16 <212> TYPE: DNA

17 <213> ORGANISM: Artificial Sequence

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19 <223> OTHER INFORMATION: pGFP+Luc\* plasmid

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25 <223> OTHER INFORMATION: Coding sequence for GFP

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28 <223> OTHER INFORMATION: coding sequence for firefly luciferase

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31 <223> OTHER INFORMATION: coding sequence for beta-lactamase

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163						43 M T /	<b>NI.</b> (	2011 20		F <b>f</b> i,	rofl:	. 3.1.	ni for	~~ ~~	unkı	Oten		
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165		<b>\400</b> >				7 l s	T.v.e	λen	Tla	T.v.c	T.vc	Glv	Dro	Δla	Dro	Phe	ጥህጕ	Pro
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178   Glu Leu Leu Asn Ser Met Asn Ile Ser Gln Pro Thr Val Val Phe Val 179   115   120   120   125   125   125   180   Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro 181   130   130   135   140   140   140   182   11e Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly 183   145   150   150   155   155   160   184   Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe 185   165   170   175   175   186   Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile 187   180   180   185   190   185   190   195   195   200   205   190   Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp 191   210   215   220   192   Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val	176		Phe	Ile	Gly	Val	Ala	Val	Ala	Pro	Ala	Asn	Asp	Ile	Tyr	Asn	Glu	Arg
179       115       120       125       125       126       127       128       128       128       128       128       128       128       128       128       128       128       140       140       140       181       130       135       140       140       140       181       140       182       112       140       140       140       182       140       1	177																	
180       Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro         181       130       135       140         182       Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly         183       145       150       155       160         184       Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe       165       170       175         186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile       187       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val         189       195       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			Glu	Leu	Leu	Asn	Ser	Met	Asn		Ser	Gln	Pro	Thr		Val	Phe	Val
181       130       135       140         182       Ile Ile Gln Lys Ile Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly       183       145       150       155       155       160         184       Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe       165       170       175       175         186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile       187       185       190       185       190       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val       189       200       205       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp       220       220         191       210       215       220       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val														_				
182       Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly         183       145       150       155       160         184       Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe       165       170       175         186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile       187       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val       189       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp       220         191       210       225       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			Ser	_	Lys	Gly	Leu	Gln		Ile	Leu	Asn	Val		Lys	Lys	Leu	Pro
183       145       55       155       160         184       Phe Gln       Ser Met       Tyr Thr       Phe Val       Thr       Ser His       Leu       Pro       Pro       Gly       Phe         185       165       165       170       170       175       175       175         186       Asn Glu       Tyr       Asp       Phe       Val       Pro       Glu       Ser       Phe       Asp       Arg       Arg       Thr       Ile         187       180       180       185       185       190       190       Leu       Pro       Lys       Gly       Val         188       Ala       Leu       Ile       Met       Asn       Ser       Ser       Gly       Ser       Thr       Gly       Leu       Pro       Lys       Gly       Val         189       195       205       205       205       205       205       190         190       Ala       Leu       Pro       His       Arg       Arg       Arg       Val       Arg       Phe       Ser       His       Ala       Arg       Arg       Arg       Arg       Arg       Arg       Arg       <			_		_							_	_		_	_		
184       Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe         185       165       170       175         186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile       187       180       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val       205       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val														Thr	Asp	Tyr	GIn	
185       165       170       175         186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile         187       180       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val         189       195       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp       Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val														<b>T</b>	D	D	<b>a</b> 1	
186       Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile         187       180       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val         189       195       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			Pne	GIn	ser	мет	_	Thr	Pne	vaı	Thr		HIS	Leu	Pro	Pro	-	Pne
187       180       185       190         188       Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val         189       195       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			n an	C1.,	Ш** <b>*</b>	N an		Wa I	Dro	C111	C02		N an	7 ~~	7 cn	Tvc		Tlo
188 Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val 189 200 205  190 Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp 191 210 215 220  192 Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			HSII	GIU	тАт	_	rne	val	F10	GIU		FIIG	vəħ	nry.	vəh		TIT	116
189       195       200       205         190       Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp         191       210       215       220         192       Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			λla	T.011	т1Д		λen	Sor	Sar	Glv		Thr	G1v	T.Q11	Dro		Glv	Va l
Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp 191 210 215 220 192 Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			та	ьeu		ric C	nou!	DET	Det	_	Det		O T Y	Leu		פונה	OT Y	7 U.I
191 210 215 220 192 Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val			Ala	Len		His	Arσ	Thr	Ala		Va1	Ara	Phe	Ser		Ala	Ara	Asp
192 Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val					0		••• 9			0,15	,	9					7	
			Pro		Phe	Glv	Asn	Gln		Ile	Pro	Asp	Thr		Ile	Leu	Ser	Val
						-						-						

194		Val	Pro	Phe	His		Gly	Phe	Gly	Met		Thr	Thr	Leu	Gly	_	Leu
195		1	_		-1	245	1			20.1	250	_	- 1	<b>~</b> 1	<b>a</b> 1	255	<b>-</b> .
196		11e	Cys	Gly		Arg	vaı	vaı	Leu		Tyr	Arg	Pne	GIU		Glu	Leu
197		-1	_		260		<b>a</b> 1.	•		265	<b>-1</b>	<b>a</b> 1	<b>a</b>		270	T	**- 1
198		Phe	Leu	Arg	ser	Leu	GIn	Asp	_	Lys	тте	GIn	ser		Leu	Leu	vaı
199		_	_,	275	-1	_	51	-1.	280		<b>a</b>	m1	<b>-</b>	285	•	<b>-</b>	
200		Pro		Leu	Pne	ser	Pne		Ата	гĀг	ser	Thr		тте	Asp	гĀЗ	Tyr
201		_	290	_	_	_	•	295	-1		<b>.</b>	~ 3	300		<b>-</b>		_
202		_	Leu	Ser	Asn	Leu		GLu	тте	Ата	ser	_	GTĀ	Ата	Pro	Leu	
203		305					310			_	_	315	'	_	_		320
204		rys	GLu	Val	GTĀ		Ата	vaı	Ата	гĀг	_	Pne	HIS	ьеи	Pro	_	тте
205		_		~ 1		325	_	_,	-1	_,	330	_		~ 1	_	335	-1
206		Arg	GIn	Gly	-	GTĀ	Leu	Thr	GIu		Thr	ser	Ата	тте		тте	Thr
207		_	<b>~</b> 3	-1	340		_		<b>a</b> 1	345		<b>a</b> 1		**. 1	350		<b>5</b> 1
208		Pro	GLu	Gly	Asp	Asp	Lys	Pro	_	Ala	vai	GIY	ьуs		vaı	Pro	Pne
209				355	_			_	360	_	_,		_	365	_		
210		Phe		Ala	Lys	Val	Val		Leu	Asp	Thr	GIY		Thr	Leu	GLY	Val
211			370				_	375	<b>-</b>	_		_	380			_	
212			Gln	Arg	GLy	Glu		Cys	Val	Arg	Gly		Met	IIe	Met	Ser	_
213		385					390					395		_	_	_	400
214		Tyr	Val	Asn	Asn		Glu	Ala	Thr	Asn		Leu	шe	Asp	Lys		GIY
215		_	_	•	_	405	_			_	410	_		_	<b>a</b> 1	415	_1
216		Trp	Leu	His		GLY	Asp	Ile	Ala	-	Trp	Asp	GLu	Asp		His	Phe
217		_,		1	420	_	_	_	_	425	-1.				430	-	<b>a</b> 3
218		Phe	He	Val	Asp	Arg	Leu	Lys		Leu	He	Lys	Tyr		GLĀ	Tyr	GIn
219				435			_	~ .	440	_,	_	_	~ 3	445		_	-1
220		Val		Pro	Ala	GIu	Leu		ser	тте	Leu	Leu		HlS	Pro	Asn	ше
221		_,	450					455	_	_	_	_	460		-1	-1	-
222			Asp	Ala	GLY	vaı		GTĀ	ьeu	Pro	Asp		Asp	Ата	GIY	GIU	
223		465			**- 1	**- 7	470	<b>.</b>	<b>~</b> 1	***	<b>a</b> 1	475	m1	37-4	m1	<b>a</b> 1	480
224		Pro	Ата	Ala	vaı		val	Leu	GIU	HIS	_	гàг	Thr	мет	Thr		rys
225		a1	<b>-1</b> -	17. 1	3	485	77- 7	31-	O	<b>01</b> =	490	mh	mh	7 1 n	T	495	T 011
226		GIU	ше	Val	_	Tyr	vaı	Ата	ser		val	Thr	Thr	Ата		гàг	Leu
227		3	<b>a</b> 1	a1	500	77 <b>-</b> 1	Dh.	37 <sub>-</sub> 1	3	505	17_1	D	T	<b>01</b>	510	m la	G.1
228		Arg	СТА	Gly	Val	vaı	Pne	vaı	_	GIU	val	Pro	гаг	_	Leu	THE	GTA
229		T *** a	Ton	515	<b>71</b> ~	7 ~~	T ***	т1.	520	C1.,	т1.	T 011	т1.	525	ת 1 ת	T	Tric
230	•	гаг		Asp	Ата	Arg	гуѕ		Arg	GIU	тте	ьeu		гуѕ	Ата	ьуѕ	гуѕ
231		<b>~1</b>	530	T	C = m	T	т	535					540				•
232 233		545	СТУ	Lys	Ser	гуѕ	550										
	<210>		TD 1	ντ <b>Ω</b>			550										
					ŧ												
	<211> <212>																
	<213>				hnor	v.m											
	<220>				וסוועו	w II											
	<223>				<i>ለ</i> አጥ ተ ⁄	`N.	301·~	70 0	F had	1.	a+	n a c c	unk	.05:75			
	<400>				JEZT T (	7N: 3	our(	JE 0.	L De	La T	ıctal	uase	uliki	TOWII			
241	~4UU>				G1 n	uic	Dho	λ ~~	W= 1	λlo	Lou	T10	Dro	Dha	Dha	λls	בות
242		met 1	ser.	Ile	GIII	5 5	rne	мту	۷ат	HId	10	тте	LTO	FIIE	rne	15	нта
243		т.				,					TO					TO	